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NEWER SCREENING TEST FOR PORPHYRIA*

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During the past several years, photosensitivity to bithionol, hexachlorophene, and salicylanilides has become more prevalent. Persons who develop photosensitivity to these chemicals, which are integral ingredients of antibacterial soaps, must be evaluated to determine whether an abnormality of porphyrin metabolism is playing a part in the photosensitivity. Photocontact sensitivity usually carries a better prognosis and is more amenable to therapy than porphyria.

The common laboratory screening test for porphyria is the Watson-Schwartz test for porphobilinogen, which is present in the urine of patients with symptomatic acute intermittent porphyria and mixed porphyria, of which only the latter is a photosensitive disease process (1). Needless to say, porphyria cutanea tarda and congenital erythropoietic porphyria (Morbus Gunther), neither of which have elevated urinary porphobilinogen, will be overlooked with only this one screening procedure. It should be noted that neither porphobilinogen nor porphyrins are excreted in excess in the urine in erythropoietic protoporphyria. In this type of photosensitive porphyria, tests for the detection of excessive protoporphyrin in the erythrocytes must be utilized (2). A test for qualitatively screening for all urinary porphyrins by utilizing an anion exchange resin in a batch process has been described in a recent article (3) from this institution. Those studies demonstrated the advantages of using an anion exchange resin to remove interfering green fluorescent materials. The present paper represents a simplification of this latter procedure by using a disposable anion exchange column.

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METHODS

A disposable plastic column (Fig. 1) charged with anion exchange resin (Dowex 1-X8, 50/100 mesh, chloride form) is used.[1] Approximately 1 ml of distilled water is poured into the reservoir of the column to wet the resin and to ensure that the column is properly packed. A 4 ml aliquot of urine (mixed well if any precipitate is present) is then poured into the reservoir of the column and allowed to drip through the anion exchange resin. The unabsorbed materials are washed through the column with 7.5 ml of distilled water (full reservoir). When the water has flowed through the column, porphyrins are eluted by the addition of two 2 ml portions of 3 N HCl (1:4 dilution of concentrated HCl) and both portions of the HCl eluate are collected in a test tube.[2] The eluate is examined under a Wood's ultraviolet light in a darkened room for the presence of fluorescence. The test is considered positive when a reddish-pink fluorescence is present. A normal urine sample is carried through the same procedure as a control.

RESULTS AND DISCUSSION

For evaluation of this qualitative test, eight simulated porphyria urine specimens were produced by the addition of porphyria urine to non-porphyric urine samples. The results of these experiments are shown in Table I. The porphyrin content of the simulated porphyria urine specimens was determined with a Beckman DU spectrophotometer (4), while that of the normal samples was determined with an Aminco-Bowman Spectrophotofluorometer. The amount of green fluorescence in urine specimens used in this study showed an 11-fold variation, which is comparable to that noted previously for hospital patients (3). The studies summarized in Table I indicate that porphyrins may be readily de-

[1] The disposable plastic columns charged with anion exchange resin were provided by Dr. Burton A. Zabin, Director of Research, Bio-Rad Laboratories, Richmond, California. Freddie L. Hill, B.S., and Elizabeth S. Head, M.T. gave technical assistance in this work.

[2] Two portions of HCl are required, since some of the absorbed porphyrins are washed off the resin into the HCl in the reservoir above the resin when the first portion of HCl is added. The second portion of HCl serves to wash the remaining porphyrins through the column.

tected in urine at levels as low as 1.5 mg/liter. Whenever the amount of green fluorescence in the sample is not abnormally high (relative green fluorescence of 50 or less), porphyrins may be detected at concentrations as low as 0.8 mg/liter. For a qualitative test, this would appear to be about the proper level of sensitivity. With very few exceptions, patients with porphyria cutanea tarda excrete porphyrins in amounts above 1 mg/liter. In normal individuals, total urinary porphyrin excretion is below 0.3 mg/24 hours. However, in certain conditions other than porphyria, uroporphyrin may rise as high as 0.5 mg/24 hours (4). Coproporphyrinuria (0.3 mg to 4 mg of coproporphyrin per 24 hours) may occur as a consequence of lead poisoning, hepatic disorders, alcoholism, etc. (4). The porphyrin screening test utilized in this study would detect some cases of coproporphyrinuria as well as porphyria cutanea tarda, erythropoietic porphyria and mixed porphyria. Consequently, more specific tests should be utilized to characterize the porphyrins when a positive porphyrin screening test is obtained. If necessary, (e.g., on dilute urine specimens) the sensitivity of the screening test for porphyrins may be increased further by increasing the amount of urine applied to the column from 4 ml to 7.5 ml (i.e., the reservoir capacity).

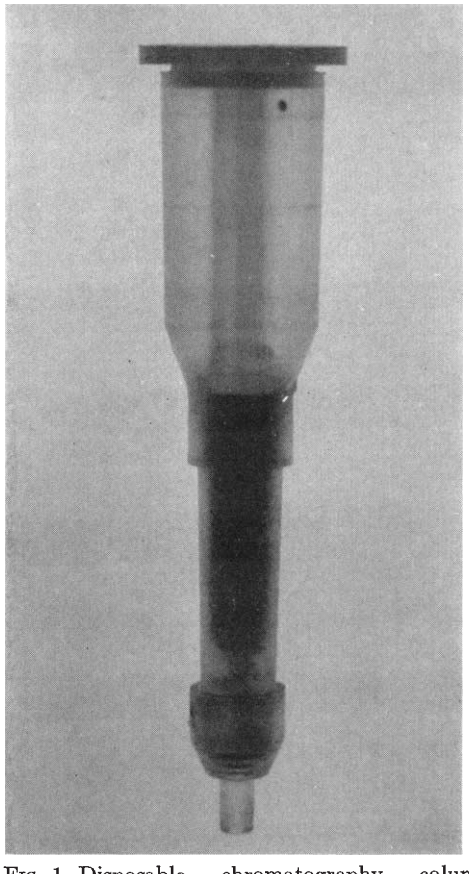

FIG. 1. Disposable chromatography column prepacked with anion exchange resin (0.6 cm × 4 cm column), as obtained from Bio-Rad Laboratories. Richmond, California.

TABLE I

Detection of porphyrins in urine samples

Experiment	No. of urine samples	Porphyrin concentration in urine (mg/liter)	Relative green fluorescence*	Results of resin screening test†
1A	4	1.5	13 to 50	all positive
1B	8	0.03–0.09	13 to 50	all negative
2A	4	1.5	29 to 112	all positive
2B	4	0.04–0.10	27 to 155	all negative
Porphyria urines	2	3.0, 4.5	40, 75	both positive

* Expressed as instrument reading at 520 nm of untreated urine diluted 1:5 with 0.5 N HCl, using an Aminco-Bowman Spectrophotofluorometer. An activation wave length of 400 nm was used, with a meter multiplier setting of 0.03, sensitivity setting of 39, with slit arrangement No. 3, and an R136 photomultiplier tube.

† Carried out as a blind test by the procedure described in the text.

This modification of the previously described anion exchange resin screening test for urinary porphyrins (3) eliminates the necessity of buying the ion exchange resin in quantities too large to be reasonably used, and it simplifies the testing procedure by utilizing disposable ion exchange resin columns which are ready to use as purchased. The flared portion of these columns sits directly on top of the test tube, so equipment requirements are minimal. The column procedure has an additional advantage over the previously described batch procedure since urinary precipitates will not interfere with the test.

These precipitates, with their adsorbed porphyrins, will be retained at the top of the column, and the eluant HCl will remove the porphyrins adsorbed to the precipitate as well as the porphyrins bound by the anion exchange resin. Therefore, urine specimens may be kept refrigerated several days prior to analysis without affecting the validity of the test.

SUMMARY

A simplified screening test for urinary porphyrins utilizing a disposable anion exchange column is described. The sensitivity of this test is equivalent to the anion exchange resin batch method screening test for urinary porphyrins but is more easily performed. Possible false negative tests by adsorption of porphyrins on urinary precipitates are eliminated. Both the batch and the column procedures eliminate most of the interfering green fluorescing materials of urine by selectively absorbing the porphyrins onto the resin.

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